JOURNAL OF BACTERIOLOGY, Mar. 1993, p. 1637–1644
0021-9193/93/061637-08$02.00/0

Vol. 175, No. 6

Cloning, Nucleotide Sequence, and Regulatory Analysis of the *Lactococcus lactis dnaJ* Gene

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The *dnaJ* gene of *Lactococcus lactis* was isolated from a genomic library of *L. lactis* NIZO R5 and cloned into pUC19. Nucleotide sequencing revealed an open reading frame of 1,137 bp in length, encoding a protein of 379 amino acids. The deduced amino acid sequence showed homology to the DnaJ proteins of *Escherichia coli*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, and *Clostridium acetobutylicum*. The level of the *dnaJ* monocistronic mRNA increased approximately threefold after heat shock. The transcription initiation site of the *dnaJ* gene was determined and appeared to be preceded by a typical gram-positive vegetative promoter sequence (TTGCCA-17 bp-TAAAAT). Upstream of the promoter region, an inverted repeat is located that is identical to those detected upstream of heat shock genes of other gram-positive organisms. A transcriptional fusion between the *dnaJ* expression signals and a *usp45-amyS* secretion cassette caused a significant increase in α-amylase activity after heat shock induction. Deletion mutagenesis showed that the inverted repeat is involved in heat shock regulation of the *dnaJ* gene. The conservation of this palindromic sequence in gram-positive heat shock genes suggests a common regulatory pathway distinct from the system used in gram-negative bacteria.

An abrupt increase in growth temperature usually causes the induction of synthesis of a small group of proteins called the heat shock proteins. This response is a common feature in eubacterial, archaebacterial, and eukaryotic organisms. Not only is the reaction to heat shock similar, but the structure and function of the induced proteins are highly conserved (for a recent review, see reference 1).

The *dnaJ* gene of *Escherichia coli* was originally discovered to be essential for bacteriophage lambda replication (39). Recently, it was demonstrated that DnaJ is also involved in the replication of phage P1 (49) and *oriC* plasmids (22). One of the major activities of DnaJ is to stimulate the ATPase activity of DnaK, the prokaryotic member of the HSP70 family. This enhanced ATPase activity may result in an efficient recycling of DnaK (20). Furthermore, DnaJ is also believed to target other proteins for action by DnaK (48). Because of its cooperation with DnaK, DnaJ also plays a role in protein folding (11) and in the facilitation of export of homologous and hybrid proteins (29, 50).

Analysis of the heat shock response of *Lactococcus lactis* has revealed the induction of 13 to 16 proteins after a shift in temperature from 30°C to 37 or 42°C (2, 47). Immunological screening of these induced proteins showed the presence of GroEL- and DnaK-like heat shock proteins in *L. lactis* (2, 47). In addition, the lactococcal counterparts of the heat shock proteins GrpE and DnaJ could also be detected (2). Recently, the *groELS* operon of *L. lactis* was cloned and its nucleotide sequence was determined (17).

In this report, we describe the cloning and characterization of the *dnaJ* gene of *L. lactis*. We show that its expression is regulated at the transcriptional level and is critically dependent on the presence of a palindromic structure immediately preceding its promoter.

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MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. *E. coli* JM83 (45) and *L. lactis* MG1363 (12) and NIZO R5 (30) were used. The plasmids used are listed in Table 1. *E. coli* was grown in TY broth (34) or on TY broth solidified with 1.5% agar. *L. lactis* was grown in glucose M17 medium (40) or in whey-permeate broth (10). For the induction of heat shock response, *L. lactis* cells were grown at 30°C to an optical density at 600 nm of 0.6. Cells were pelleted by centrifugation and resuspended in whey-permeate broth at 30, 37, or 42°C and incubated for 10, 20, or 30 min at those temperatures. For electroporation of *L. lactis*, cells were cultured, washed, and recovered as described previously (15) and plated on glucose M17 agar plates. The antibiotics used for selection in media were chloramphenicol (10 μg/ml) and ampicillin (50 μg/ml).

DNA manipulations. Plasmid DNA was isolated as described previously (4). For *L. lactis* cells, TMS buffer (43) containing 2% lysozyme was used for 30 min at 37°C to generate protoplasts. Enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, Md.) and New England Biolabs Inc. (Beverly, Mass.) and were used as recommended by the suppliers. DNA manipulations were performed essentially as described previously (35).

Cloning of the *dnaJ* gene and immunological methods. A genomic library of *L. lactis* NIZO R5 partial *Sau*3A fragments was prepared in *E. coli* MB406 (Promega, Madison, Wis.) by using the EMBL arms cloning system (Packagene Lambda Packaging System; Promega, Madison, Wis.) as described previously (33). Cellular extracts of *L. lactis* NIZO R5 were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (18). A mixture of proteins with molecular masses of approximately 40 kDa, including glyceraldehyde-3-phosphate dehydrogenase, was excised from the gel and recovered by isotachophoresis (27). Antibodies against this partially purified protein fraction were raised and were used to screen the genomic library. Immunoblotting was performed as described previously (42). Screening of the

TABLE 1. Plasmids used in this study

Plasmid	Relevant genetic characteristics[a]	Source or reference
pUC19	Cloning vector; Ap^r	45
M13 mp18 and 19	Cloning vector for sequencing purposes	23
pNZ2015	pUC19 carrying an *StyI-XhoI* fragment containing the *dnaJ* gene; Ap^r	This study
pNZ2016	pUC19 carrying an *SphI-XhoI* fragment containing the *dnaJ* gene; Ap^r	This study
pNZ10α5	pNZ123 carrying the *amyS* gene fused to positions −158 to 127 of the *usp45* gene; Cm^r	43
pNZ20α1	pNZ123 carrying the *usp45-amyS* cassette fused to positions −217 to 41 of the *dnaJ* gene; Cm^r	This study
pNZ20α3	pNZ123 carrying the *usp45-amyS* cassette fused to positions −40 to 41 of the *dnaJ* gene; Cm^r	This study

[a] Abbreviations: Cm, chloramphenicol; Ap, ampicillin.

library, preparation of liquid lysates, and DNA isolation of positive recombinant phages were performed as described previously (44).

DNA sequence analysis and evaluation of data. Restriction fragments of pNZ2015 (Table 1) were inserted into the appropriate sites of M13 mp18 or mp19 (23). DNA sequencing by the dideoxy chain method (36) was performed with Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) and the universal M13 primer or oligonucleotides synthesized with a Cyclone DNA synthesizer (Biosearch, San Rafael, Calif.). The DNA sequence was analyzed with the PC/GENE software system (IntelliGenetics Inc., Geneva, Switzerland). The data base search was performed with the CaosCamm facilities in Nijmegen, The Netherlands (9).

RNA analysis. After 10 min of induction at 30, 37, or 42°C, 25 ml of cells was pelleted by centrifugation and immediately frozen in liquid nitrogen. After resuspension in 0.5 ml of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA), total RNA was isolated by using macaloid clay (32). After addition of 0.6 g of zirconium beads (0.1 mm in diameter; Biospec Products, Bartlesville, Okla.), 0.17 ml of 4% macaloid clay suspension, 0.5 ml of phenol and 50 μl 10% sodium dodecyl sulfate, cells were disrupted in a bead beater (Biospec Products). After centrifugation, a phenol-chloroform extraction was performed. The RNA was precipitated and stored at −80°C. Northern (RNA) blot analysis was performed as described previously (31).

Primer extension analysis. A synthetic oligonucleotide complementary to positions 3 to −27 of the *dnaJ* gene was used in a primer extension experiment. Primer (1 pmol) was annealed to 30 μg of RNA and the cDNA synthesis was performed as previously described (8). The product was analyzed on a 6% polyacrylamide-urea sequencing gel together with a dideoxy sequencing reaction with the same primer.

Construction of a *dnaJ-amyS* transcriptional fusion. A transcriptional fusion between the *dnaJ* expression signals and a *usp45-amyS* gene fusion (43) was constructed. For this fusion, a recombinant polymerase chain reaction (PCR) protocol (16) was adjusted (Fig. 1). Three primers were used: (i) a fusion primer with the sequence 5′-GGAAGTGAGTAAT TTAGAAATGAAAAAAAAGATTATCTCAGC-3′, of which the 5′ end was complementary to positions 23 to 44 of *dnaJ* and the 3′ end was complementary to the first 23 nucleotides of the *usp45* signal sequence; (ii) an oligonucleotide with the sequence 5′-CGACTTCGGGATGATCC-3′, complementary to the *amyS* gene; and (iii) the reverse sequencing primer (New England Biolabs Inc.). Plasmids pNZ10α5 (Table 1) (43), which contained the *usp45-amyS* fusion encoding the Usp45 signal peptide fused to the mature α-amylase of *Bacillus stearothermophilus*, and plasmid pNZ2016 (Table 1) were used as templates. The three primers and two templates were used simultaneously in one PCR with annealing at 46°C. In the first 2 cycles, a product was generated from the fusion primer, the α-amylase primer, and pNZ10α5 (Fig. 1A and B). The 3′ end of this product was complementary to the fusion primer and served as a primer on pNZ2016 (Fig. 1C). The generated product was further amplified with the reverse primer (Fig. 1D) and the α-amylase primer. After 30 cycles of PCR, the two expected products were found. One was the product of the fusion primer and the α-amylase primer with pNZ10α5 as the template, and the other was a fragment containing the *dnaJ* expression signals fused to the *usp45-amyS* fusion (Fig. 1E). Subsequently, the fusion product was cut with *XbaI* and *SstII* and ligated into pNZ10α5 digested with the same enzymes, resulting in pNZ20α1. For the construction of pNZ20α3, pNZ20α1 DNA served as a template in a PCR with the α-amylase primer and a primer complementary to positions −40 to −14 of the *dnaJ* gene, preceded by an *XbaI* site (5′-GGGTCTAGATTTTTTTGCC AAAAATGAAAAAACGTG-3′). The product was cut with *XbaI* and *SstII* and ligated into pNZ10α5 digested with the same enzymes.

α-Amylase activity assay. Culture supernatant was incubated with 20 mg of amylose azure (Sigma Chemical Co, St. Louis, Mo.) for 60 min at 60°C in 1 ml of α-amylase buffer (50 mM Tris-HCl [pH 7.5], 50 mM NaCl, 5 mM $CaCl_2$). After centrifugation, A_{595} of the supernatant was measured.

Nucleotide sequence accession number. The DNA sequence shown in Fig. 2 has been assigned GenBank data base accession no. M99413.

RESULTS

Isolation of the *dnaJ* gene from *L. lactis* NIZO R5. A genomic library of strain R5 was screened with antibodies raised against intracellular proteins of *L. lactis* approximately 40 kDa in size. This screening resulted in the isolation of four recombinant phages. As expected, analysis of phage lysates of these recombinant phages by immunoblotting revealed that they all directed the synthesis of proteins approximately 40 kDa in size. Analysis of the DNAs of the phages showed that they contained inserts 12 to 19 kb in length. Restriction endonuclease mapping revealed an 8-kb *XhoI-KpnI* fragment present in all inserts. This fragment was used for further analysis. Different overlapping fragments were inserted into pUC19. Immunoblot analysis of *E. coli* JM83 harboring these plasmids showed that a 2-kb *StyI-XhoI* DNA fragment directed the synthesis of the 40-kDa protein. The plasmid containing this fragment was designated pNZ2015.

Nucleotide and encoded amino acid sequences. The nucleotide sequence of the *StyI-XhoI* fragment of pNZ2015 was determined (Fig. 2). The largest open reading frame (ORF)

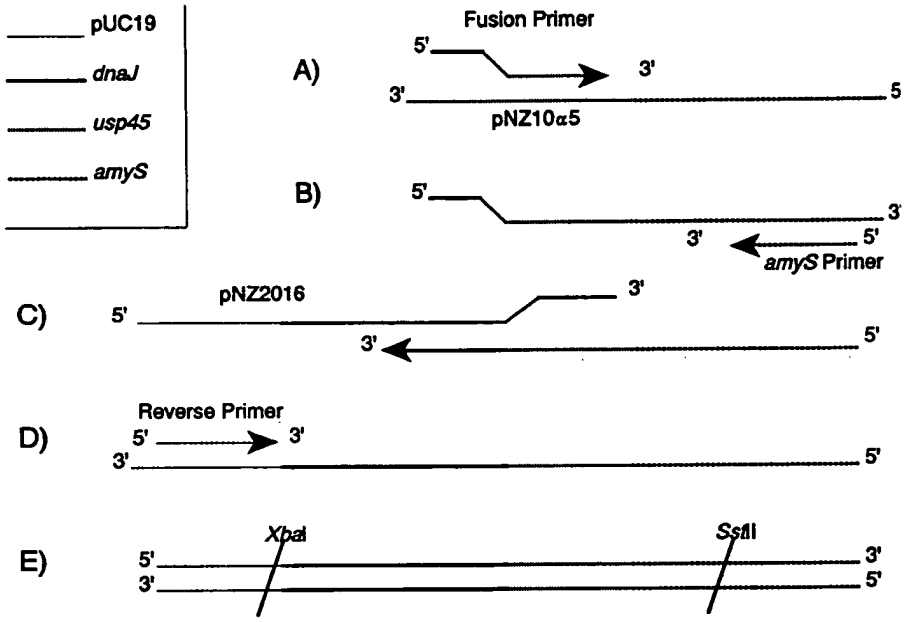

FIG. 1. Schematic drawing of the recombinant PCR method for the construction of a transcriptional fusion of the *dnaJ* expression signals with the *usp45-amyS* fusion as described in Materials and Methods. Only steps involved in the production of the fusion and their products are displayed. Annealing of the first (A), second (B), third (C), and fourth (D) cycles and the end product (E) are shown.

present on this fragment was ORF1, with a size of 1,173 bp. ORF1 could encode a 379-residue protein with a calculated molecular mass of 40,786 Da. This molecular mass is in agreement with the size of the protein reacting with the antibodies used. The deduced amino acid sequence of the protein shows a high glycine content (16%) and contains four repeats of a motif consisting of CXXCXGXG (residues 154 to 161, 171 to 178, 197 to 204, and 211 to 218). Furthermore, the sequence GGFGG is repeated three times in the N-terminal part (residues 75 to 79, 80 to 84, and 96 to 100). ORF1 is preceded by a Shine-Dalgarno sequence (37) at position 20 for which a free energy of −11.8 kcal/mol could be calculated (41). A second ORF (ORF2) at least 200 bp in length terminates at position −257; only the 3′-terminal part of ORF2 is present on the *Sty*I-*Xho*I fragment. Downstream of ORF1, no ORFs larger than 133 bp are present.

An inverted repeat (IR) extending from positions −69 to −39 is located between ORF1 and ORF2. Downstream of ORF1, no sequences with significant secondary structures were found.

Relationship of the determined ORFs to other amino acid sequences. The deduced amino acid sequence of ORF1 was compared with sequences in the NBRF-Pir (version 32.0) and SWISS-PROT (version 21.0) data bases and published protein sequences. This analysis revealed the high degree of similarity of the encoded protein to several bacterial DnaJ heat shock proteins: DnaJ from *E. coli* (3, 28) (45.2% identity), *Mycobacterium tuberculosis* (19) (37.5% identity), and *Bacillus subtilis* (46) (57% identity) (Fig. 3). In addition, similarity to the published N-terminal sequence of DnaJ of *Clostridium acetobutylicum* (51% identity) (25) was found. The amino acid sequence also shared significant homology with SIS1 (21), YD1 (6), and SCJ1 (5), three eukaryotic counterparts of this heat shock protein from *Saccharomyces cerevisiae*. Alignment of the proteins revealed the conservation of the CXXCXGXG repeats. From these data, together with the heat shock regulation data described below, we conclude that ORF1 encodes the lactococcal *dnaJ* gene. Analysis of the homology of ORF2 and the small ORFs downstream of ORF1 revealed no significant homology with known proteins.

Transcriptional analysis of the *L. lactis dnaJ* gene. The start of transcription of the *dnaJ* gene was determined by primer extension analysis of RNA isolated at 30°C or after heat shock (Fig. 4A). These experiments revealed that the transcription initiation starts at an adenine at position 1. This start of transcription is preceded by the sequence TTGCCA-17 bp-TAAAAT (positions −35 to −7), thus resembling the consensus sequences for vegetative gram-positive promoters (13). The putative −10 region is preceded by the sequence TGN, which is also present in more than 50% of the lactococcal promoters determined so far (38). In RNA isolated from a 30°C culture, a small amount of cDNA could be observed. In equal amounts of RNA isolates from cultures after heat shock, the quantity of primer extension product increased approximately two- to threefold with respect to 30°C, indicating an elevated amount of transcripts after heat shock. Furthermore, no additional transcription initiation sites could be detected under these conditions.

Northern blot analysis was performed by use of a radioactively labeled *Hpa*I-*Xho*I fragment containing the part of the *dnaJ* gene downstream of position 390. Analysis of RNA isolated from *L. lactis* NIZO R5 grown at 30°C showed that the *dnaJ* gene was transcribed as a 1.8-kb mRNA (Fig. 4B). The amount of mRNA increased approximately twofold after a heat shock of 37°C and three- to fourfold after a heat shock of 42°C. A 1.0-kb product also hybridized with the probe. When a probe complementary to the 5′ end of the *dnaJ* gene was used, this product could not be detected, indicating that it probably represents a 3′-terminal breakdown product of the *dnaJ* mRNA (results not shown). No large products were detected after heat shock.

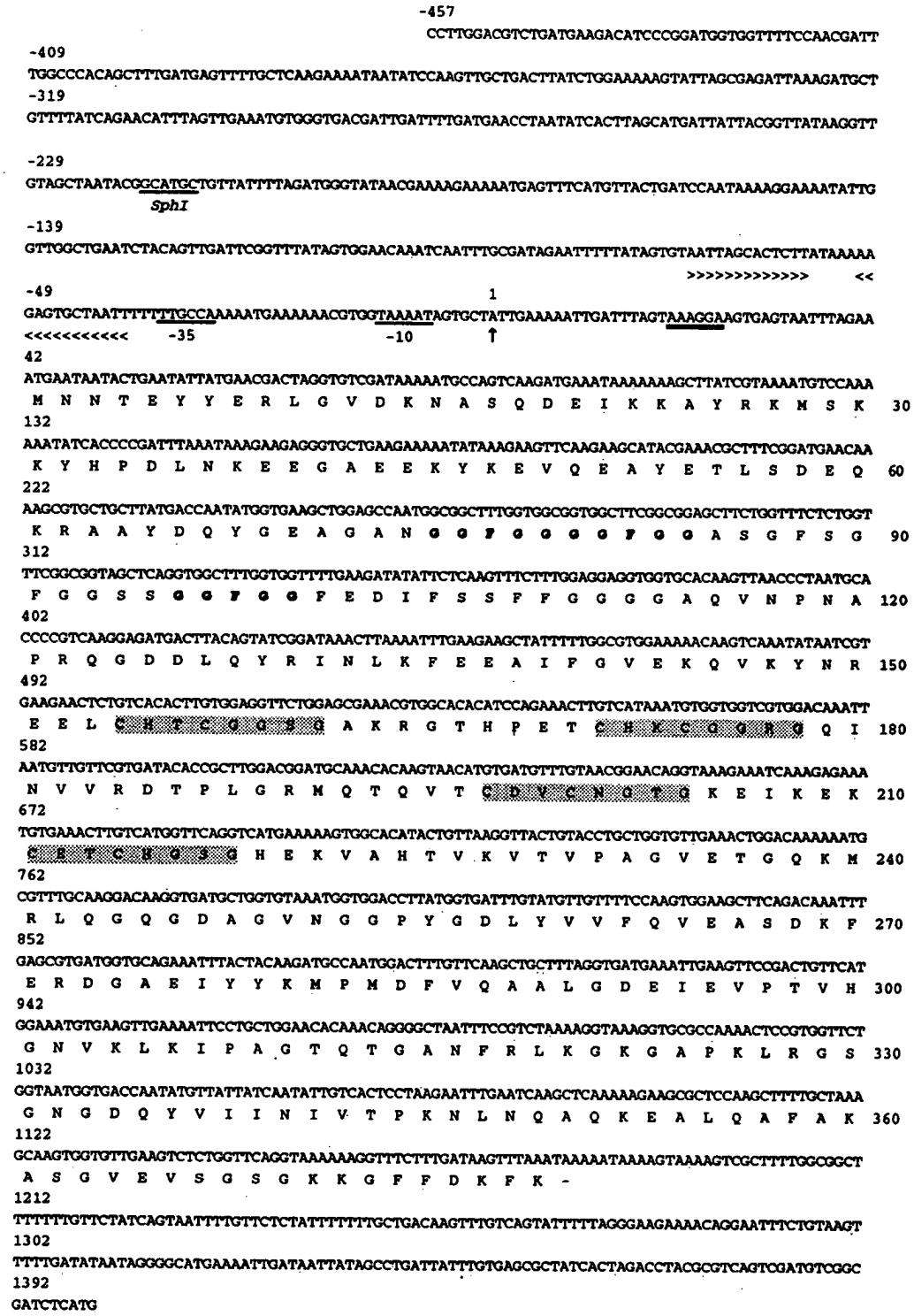

FIG. 2. Nucleotide and deduced amino acid sequence of the *dnaJ* gene of *L. lactis*. The putative Shine-Dalgarno sequence (double-underlined), the −10 and −35 sequences (underlined), and the IR (arrowheads below the sequence) are indicated. The 5′ end of the mRNA, as identified by primer extension, is marked with an arrow. The repeated CXXCXGXG motif in the protein sequence is shaded, and the repeated GGFGG sequences are in boldface.

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L1   MNNTE-----YYERLGVDKNASQDEIKKAYRKMSKKYHPDLNK-EEGAEE  44
Bs   MSKRD-----YYEVLGVSKSASKDEIKKAYRKLSKKYHPDINK-EAGSDE  44
Ec   MAKQD-----YYEILGVSKTAEEREIKKAYKRLAMKYHPDRNQGDKEAEA  45
Mt   MAQREWVEKDFYQELGVSSDASPEEIKRAYRKLARDLHPDANPGNPAAGE  50

L1   KYKEVQEAYETLSDEQKRAAYDQYGEAG-ANGGFGGGGFGGA--SGFSGF  91
Bs   KFKEVKEAYETLSDDQKRAHYDQFGHTD-PNQGFGGGGFGGG---DFGGF  90
Ec   KFKEIKEAYEVLTDSQKRAAYDQYGHAAPEQGGMGGGGFGGG--ADFSDI  93
Mt   RPKAVSEAHNVLSDPAKRKEYDE-TRRLPAGGGFGGRRFDSGFGGGFGGF  99

L1   GGSSGGFGG----------------FEDIFSSFFGGGGAQVNPNAPRQGD 125
Bs   G-----------------------FDDIFSSIFGGGTRRRDPKLRARGA 116
Ec   -------------------------PGDVPGDIFGGG---RGRQRAARGA 115
Mt   GVGGDGAEFNLNDLFDAASRTGGTTIGDLFGGLFGRGGSAR-PSRPRRGN 148

L1   DLQYRINLKFEEAIFGVEKQVKYNREELCHTCGGSGAKAGTHPETCHKCG 175
Bs   DLQYTMTLSFEDAAFGKETTIEIPREETCETCKGSGAKPGTNPETCEHCG 166
Ec   DLRYNMELTLEEAVRGVTKEIRIPTLEECDVCHGSGAKPGTQPQTCPTCH 165
Mt   DLE-TETLDFVEAAKGVAMPLRLTSPAPCTNCRGSGARPGTSPKVCPTCN 197

L1   GKGQQINVVRDTPLGRMQTQVTCDVCNGTGKEIKEKCETCHGSGHEKVAHT 225
Bs   GSGQLNVEQNTPFGKVVNRRVCHHCDGTGKIIKNKCADCGGKGKIKKRKK 216
Ec   GSGQQVQMRQ----GFFAVQQTCPHCQGRGTLIKDPCNKCHGHGRVERSKT 211
Mt   GSGVIN-RNQGAPGF---SEPCTDCRGSGSIIEHPCEECKGTGVTTRTRT 243

L1   VKVTVPAGVETGQKMRLQGQGDAGVNGGPYGDLYVVFQVEASDKFERDGA 275
Bs   INVTIPAGVDDGQQLRLSGQGEPGINGG-LPDLFVVFHVRAHEFFERDGD 265
Ec   LSVKIPAGVDTGDRIRLAGEGEAGEHGAPAGDLYVQVQVKQHPIFEREGN 261
Mt   INVRIPPGVEDGQRIRLAGQGEAGLRGAPSGDLYVTVHVRPDKIFGRDGD 293

L1   EIYYKMPMDFVQAALGDEIEVPTVHGNVKLKIPAGTQTGANFRLKGKGAP 325
Bs   DIYCEMPLTFAQAALGDEVEVPTLHG--KVKIPAGTQTGTKFRLRGKGVQ 313
Ec   NLYCEVPINFAMAALGGEIEVPTLDGRVKLKVPGETQTGKLFRMRGKGVK 311
Mt   DLTVTVPVSFTELALGSTLSVPTLDGTVGVRVPKGTADGRILRVRGRVCP 343

L1   KLRGSGNGDQYVIINIVTPKNLNQAQKEALQAFAKASGVEVSQSGK---K 372
Bs   NVRGYGQGDQHIVVRVVTPTNLTDKQKDIIREFAEVSG-NLPDEQE---M 359
Ec   SVRGGAQGDLLCRVVVETPVGLNERQKQLLQELQESFGGPTGEHNSPRSK 361
Mt   SAVGVAATYLSP                                      355

L1   GFFDKFK-------- 379
Bs   SFFDKVKRAFKG--D 372
Ec   SFFDGVKKPFDDLTR 376
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FIG. 3. Alignment of the deduced amino acid sequences of the DnaJ proteins of *L. lactis* (*L1*), *B. subtilis* (*Bs*) (46), *E. coli* (*Ec*) (3, 28), and *M. tuberculosis* (*Mt*) (19). Identical amino acids are indicated by asterisks, conserved residues are indicated by points (14), gaps to obtain maximum fit are indicated by dashes, and the conserved CXXCXGXG repeats are shaded.

A palindromic DNA structure is involved in heat shock regulation of *dnaJ*. The IR found at position −69 shows similarity to palindromic structures that are located at corresponding positions upstream of the heat shock genes of *B. subtilis*, *C. acetobutylicum*, *Synechocystis* sp., *Synechococcus* sp., *Mycobacterium* sp., and *Chlamydia psittaci* (46). It has been postulated that this IR could be involved in the temperature-sensitive regulation of transcription of these heat shock genes. To address this hypothesis, a construction was made in which the DnaJ-encoding region was exchanged with a *usp45-amyS* gene fusion encoding the *B. stearothermophilus* mature α-amylase preceded by the *usp45* signal peptide (43). In plasmid pNZ20α1, the *usp45-amyS* fusion is preceded by a region of *dnaJ* including positions −217 to 44 which contain the IR (Fig. 2). In plasmid pNZ20α3, only positions −40 to 44 of the *dnaJ* gene are present, and hence the IR is deleted. After introduction of pNZ20α1 or pNZ20α3 in *L. lactis* MG1363, α-amylase activities were measured 10, 20, or 30 min after heat shock at 37 or 42°C (Fig. 5). The final optical density of the cells after heat shock induction at 37°C or 42°C was not higher than the optical density of cells grown at 30°C at the indicated times. Strain MG1363 harboring pNZ20α1 resulted in two to four times higher α-amylase production after heat shock induction than before heat shock induction. However, strain MG1363 harboring pNZ20α3 showed a constitutive α-amylase production at 30°C and at elevated temperatures. The level of α-amylase production of this strain was comparable to that of MG1363 harboring pNZ20α1 after heat shock induction.

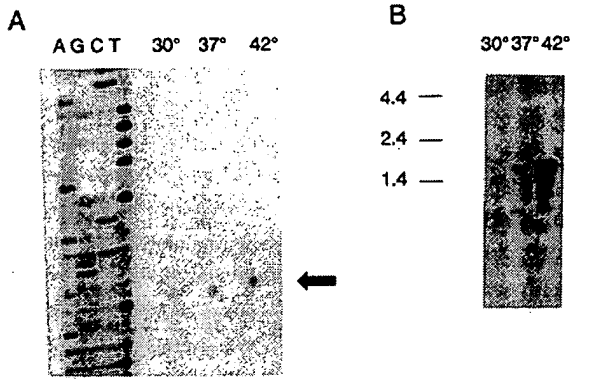

FIG. 4. Transcriptional analysis of the *dnaJ* gene. (A) Autoradiogram of the sequence gel used to analyze the primer extension products of RNA of NIZO R5, isolated after 10 min of heat shock induction at the indicated temperatures. The primer extension product is indicated (arrow). The sequence ladder obtained with the same primer is also shown. (B) Autoradiogram of a Northern blot of total RNA, isolated at 30°C or after heat shock, hybridized with a radioactively labeled *HpaI-XhoI* fragment of the *dnaJ* gene. The 1.8-kb mRNA is indicated (arrow). The sizes of the RNA markers (Bethesda Research Laboratories) are shown in kilobases.

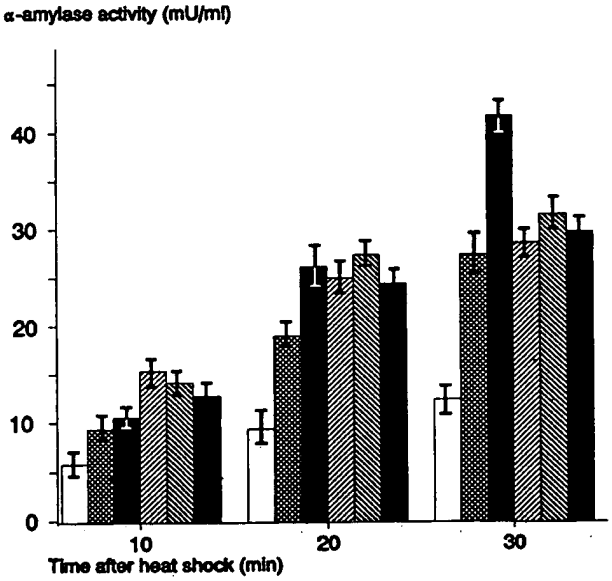

FIG. 5. Relative amounts of α-amylase activity produced by *L. lactis* MG1363 carrying a transcriptional *dnaJ-amyS* fusion. pNZ20α1 contains the *dnaJ* promoter region including the IR, while in pNZ20α3, the IR was deleted. Heat shock was induced as described in Materials and Methods. Samples were taken after the times indicated, and newly secreted α-amylase activity was assayed. MG1363(pNZ20α1) at 30°C (□), 37°C (▩), and 42°C (■) and MG1363(pNZ20α3) at 30°C (▨), 37°C (▧), and 42°C (▨) are shown.

DISCUSSION

In this report, the cloning, sequencing, and characterization of the *dnaJ* gene of *L. lactis* are described. Besides homology of the encoded protein with the DnaJ proteins of *E. coli* (3, 28) and *M. tuberculosis* (19), identity with the DnaJ proteins from *B. subtilis* (46) and *C. acetobutylicum* (25) was found. The Met residue at position 1 fits perfectly in the alignment of the different DnaJ proteins. Alignment of the reported proteins revealed a significant overall homology and the conservation of a motif consisting of CXXCXGXG, which is repeated four times (Fig. 3). This motif was also found in the eukaryotic homologs of the DnaJ proteins: SCJ1 (5), YD1 (6), and SIS1 (21). The biological meaning of this motif is not yet clear. The organization into two larger repeats, $CXXCXGXG(X)_8CXXCXGXG$, as in *YDJI* (6), was not found in the other reported proteins and is unlikely to be characteristic for DnaJ proteins. The conservation of the GGFGG sequence is less significant. Only one of the three lactococcal copies of this sequence is present in the bacterial DnaJ of *E. coli* and *B. subtilis*.

In prokaryotes, most *dnaJ* genes characterized so far are preceded by *dnaK*, which encodes another heat shock protein that is conserved among prokaryotes and eukaryotes (3, 25, 28, 46). Upstream of the *L. lactis dnaJ* gene, another ORF, designated ORF2, was found, but its deduced amino acid sequence shared no homology with known DnaK proteins, suggesting another genomic organization of these heat shock genes in *L. lactis*. The *M. tuberculosis dnaJ* gene is located 788 bp downstream of the *dnaK* gene (19). This intergenic distance exceeds the length of DNA region sequenced from *dnaJ* from *L. lactis*; hence, a conservation in genetic organization of the *dnaK* and *dnaJ* genes between *M. tuberculosis* and *L. lactis* cannot be totally excluded. The possibility that the *dnaK* gene is situated downstream of the *dnaJ* gene or elsewhere on the chromosome is also conceivable.

In *B. subtilis* (46), *C. acetobutylicum* (25), and *E. coli* (3, 28), *dnaJ* is located in an operon that also includes *dnaK*. In *L. lactis*, however, the start of transcription of *dnaJ* is located immediately upstream of the *dnaJ* gene. In addition, the size of the RNA messenger is 1.8 kb. This is too small to contain both genes. From these data, it can be concluded that the lactococcal *dnaJ* and *dnaK* genes are not organized in a single operon. The same holds for the *dnaK-dnaJ* gene organization of *Synechocystis* sp. (7). Transcriptional analysis of the *dnaK* gene of this organism revealed that it is transcribed as a monocistronic messenger. Hence, a putative *dnaJ* gene will also be present on a separate transcriptional unit.

The induction of expression of the *dnaJ* gene by heat shock was determined by three methods. First, the primer extension carried out with RNA isolated at 30°C, or after heat shock at 37 and 42°C, demonstrated a significant increase in *dnaJ* mRNA. Second, Northern blot analysis showed a twofold increase in the amount of *dnaJ* RNA after heat shock at 37°C. The amount of mRNA was even higher after heat shock at 42°C. These results confirm that the heat shock response is controlled at the transcriptional level. The same has been found for the heat shock genes of other gram-positive bacteria such as *B. subtilis* (46) and *C. acetobutylicum* (24, 25). Third, the fusion between the *dnaJ* promoter region and a *usp45-amyS* cassette caused a significant increase in α-amylase production after heat shock. Similar results were obtained in a comparable experiment in *B. subtilis* (46) with a transcriptional fusion between the *dnaK* promoter and the *amyL* gene.

Analysis of the transcription initiation site of the *dnaJ* gene revealed that it was preceded by gram-positive vegetative −10 and −35 sequences (13). The IR, located upstream of the −35 sequence, was also found upstream of the heat shock genes characterized thus far in gram-positive organisms, such as the *groELS* operons from *L. lactis* (17) and *C. acetobutylicum* (24) and the *dnaK* operons from *B. subtilis* (46) and *C. acetobutylicum* (25). Furthermore, an IR with the same sequence is located upstream of the heat shock genes of *Synechocystis* and *Synechococcus* spp. and *C. psittacci* (46). However, the IR is, at least partially, as in *Synechococcus* sp., or entirely, as in *B. subtilis* or *C. acetobutylicum*, located on the 5′ end of the mRNA in these operons, whereas in the *L. lactis dnaJ* gene, it is located upstream of the start of transcription. The IR at position −69 of the lactococcal *dnaJ* gene shares complete identity with the consensus sequence, as proposed by Wetzstein et al. (46). To examine the function of this IR in heat shock regulation, pNZ20α1 and pNZ20α3 were constructed. These plasmids contain a *usp45-amyS* fusion preceded by the promoter region of the *dnaJ* gene. In MG1363 harboring pNZ20α1, which contains the IR, the level of α-amylase activity is two to four times higher after heat shock than before heat shock. MG1363 harboring pNZ20α3, in which the IR has been deleted, showed no heat shock induction of α-amylase production. These results indicate a major role for the IR in the heat shock regulation of the *dnaJ* gene of *L. lactis*.

In transcription of *E. coli* heat shock genes, a specific sigma factor (σ^{32}) that recognizes a promoter sequence deviant from the vegetative −35 and −10 sequences (26) is involved. In the heat shock genes from *B. subtilis* (46) and *C. acetobutylicum* (24, 25), the transcription start sites are preceded by vegetative promoter sequences. The function of the IR in the heat shock regulation of the *dnaJ* gene of *L. lactis* and its conservation in sequence and location in heat shock genes of gram-positive bacteria strongly suggest a significant difference in heat shock regulation between *E. coli* and gram-positive organisms. Moreover, in *Synechocystis* and *Synechococcus* spp. and *C. psittacci*, both an *E. coli* heat shock consensus promoter sequence and the IR are present in the promoter region, suggesting that the IR is not specific for gram-positive heat shock genes. However, in the *L. lactis dnaJ* gene, the IR is unlikely to protect against RNA degradation, as suggested for *B. subtilis* by Wetzstein et al. (46), because it is located upstream of the start of transcription. For the same reason, it is unlikely to cause a pause in the production of RNA, as proposed by Narberhaus and Bahl (24). The amount of α-amylase produced by MG1363 harboring pNZ20α3 at all tested conditions was comparable with the amount produced by MG1363 harboring pNZ20α1 after heat shock. This constitutive high level of α-amylase production by MG1363 harboring pNZ20α3 suggests that the repeat is a target for a repressor, the activity of which is disturbed after heat shock. However, to be conclusive about this hypothesis, further analysis of the system is required.

ACKNOWLEDGMENTS

We are grateful to I. van Alen-Boerrigter for providing a sample of the R5 genomic library. We also express thanks to C. A. Batt for sharing unpublished results on the characterization of the *groELS* operon of *L. lactis* and W. Schumann for sharing unpublished results on the palindromic structure found upstream of the *B. subtilis dnaK* operon. We thank O. Kuipers, P. Bruinenberg, R. Siezen, and R. van Rooijen for critical reading of the manuscript.

We thank Joop Mondria, Simon van der Laan, and Henk van Brakel for artwork and photography.

This work was partly financed by the Mesdag Foundation, Leeuwarden, The Netherlands.

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